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Characterization of Sulfonylurea Receptors in Isolated Human Pancreatic Islets

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Abstract Current information on pancreatic islet sulfonylurea receptors has been obtained with laboratory animal pancreatic β cells or stable β-cell lines. In the present study, we evaluated the properties of sulfonylurea receptors of human islets of Langherans, prepared by collagenase digestion and density-gradient purification. The binding characterisitics of labeled glibenclamide to pancreatic islet membrane preparations were analyzed, displacement studies with several oral hypoglycemic agents were performed, and these latter compounds were tested as for their insulinotropic action on intact human islets. [^{3}H]glibenclamide saturable binding was shown to be linear at ≤0.25 mg/ml protein; it was both temperature and time dependent. Scatchard analysis of the equilibrium binding data at 25°C indicated the presence of a single class of saturable, high-affinity binding sites with a K_d value of 1.0 ± 0.07 nM and a Bmax value of 657 ± 48 fmol/mg of proteins. The displacement experiments showed the following rank order of potency of the oral hypoglycemic agents we tested: glibenclamide = glimepiride > tolbutamide > chlorpropamide ≫ metformin. This binding potency order was parallel with the insulinotropic potency of the evaluated compounds. J. Cell. Biochem. 71:182–188, 1998. © 1998 Wiley-Liss, Inc.

Key words: human islets; insulin release; sulfonylurea receptors; oral antidiabetic compounds

Sulfonylurea drugs are insulin secretagogues widely used in the treatment of the non-insulin-dependent diabetes mellitus (NIDDM) [Grodsky et al., 1972; Duckworth et al., 1972; Lebovitz et al., 1977]. The hypoglycemic effect of these compounds is related to the stimulation of insulin release from the endocrine pancreas [Yalow et al., 1960].

At the molecular level, sulfonylureas are proposed to stimulate insulin secretion by binding to a receptor protein on the plasma membrane of pancreatic islet β cells [Boyd et al., 1988, 1991; Kaubisch et al., 1982; Geisen et al., 1985; Schmid-Antomarchi et al., 1987; Gaines et al., 1988; Panten et al., 1989]. Receptor occupancy inhibits K^+ efflux via a plasma membrane ATP-regulated K^+ channel identified in laboratory animal islet cells [Sturgess et al., 1985], and β-cell lines such as RINm5F [Schmid-Antomarchi et al., 1987] or HIT T15 [Niki et al., 1989]. This channel participates in the control of insulin release by glucose [Aschroft et al.,1984] via changes in the intracellular [ATP]/[ADP] ratio. Subsequent depolarization of the plasma membrane causes opening of voltage-dependent Ca^{2+} channels and the rise in intracellular Ca^{2+} triggers insulin release [Gylfe et al., 1984]. Although this chain of events couples the insulin-releasing effect of sulfonylureas to their affinity to the β-cell binding sites, the precise molecular mechanism of these events remain still unknown [Yalow et al., 1960]. The fine binding characteristics of tritiated or iodinated sulfonylureas (especially glibenclamide and its analogues) have been investigated in rodent islets and β cells such as RINm5F and HIT T15 cells [Aguilar-Bryan et al., 1990; Schmid-Antomarchi et al., 1987; Panten et al., 1989; Gaines et al., 1988].

Specific binding of sulfonylurea drugs to isolated tumor β cells and membranes has been attributed in some cases to high (K_d = 0.1–1 nM) and low (K_d = 100–400 nM) affinity binding sites [Niki et al., 1989, 1990; Schmid-Antomarchi et al., 1987; French et al., 1990,

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1991; Geisen et al., 1985; Gaines et al., 1988]. These might be due to the presence of at least two subunits of 140 and 65 kDa, which may bind different sulfonylureas with different binding affinity and kinetic parameters, as shown by photo-affinity labeling studies [Aguilar-Bryan et al., 1990; Kramer et al., 1995, 1994].

Conversely, a single population of high-affinity sulfonylurea binding sites in membranes from normal rat islets, insulinoma cells, and microsomes from mice islets has been previously reported [Ozanne et al., 1995]. These latter findings seem more consistent with the pharmacodynamics of sulfonylureas. Indeed, the free plasma glibenclamide levels effective on insulin secretion and the in vitro drug concentrations required for half maximal stimulation of insulin release from rodent pancreatic islets [Panten et al., 1989] and insulin-secreting cell lines [Aguilar-Bryan et al., 1990; Schmid-Antomarchi et al., 1987] (both in the low nanomolar range) point to the high-affinity binding site as the only functional receptor, the occupancy of which ultimately leads to insulin secretion. Surprisingly, so far no information is available on the characteristics of sulfonylurea receptors on human pancreatic islet cells. Therefore, we considered it of interest to study the binding characteristics of labeled glibenclamide to pancreatic islet cell membrane preparations, and to perform displacement studies with several oral hypoglycemic agents. In addition, the insulinotropic action of these latter compounds was tested on the intact human islets.

MATERIALS AND METHODS

Preparation of the Islets

The procedures for the preparation of the islets were based on collagenase digestion and density-gradient purification, as previously described [Marchetti et al., 1994, 1995; Giusti et al., 1997]. In this study, we used islets from the pancreases of six human cadaver donors, three males and three female, aged 24–45 years. All the protocols had been approved by our local Ethics Committee.

Membrane Preparation

Membranes were prepared from human islets of Langerhans by modification of the method of Gaines et al. [1988]. Briefly, after washing twice with phosphate-buffered saline (PBS), pH 7.4, cells were suspended in 10 ml of 5 mM Tris buffer (pH 8.0 with HCl) supplemented with protease inhibitor (phenylmethanesulfonylfluoride [PMSF], 1 mM; soybean trypsin inhibitor, 20 μg/ml; benzamidine, 160 μg/ml; bacitracin, 200 μg/ml), and then homogenized with an Ultra-Turrax.

The homogenate was centrifuged at 48,000*g* for 15 min at 4°C and the supernatant was discarded. This washing step was repeated twice. The pellets from the second centrifugation were resuspended in 50 mM Mops (pH 7.4 with NaOH), 0.1 mM $CaCl_2$, rehomogenized, and centrifuged at 48,000*g* for 15 min at 4°C.

The resulting pellet was resuspended at concentration of 0.1–0.2 mg of protein/ml in buffer containing 50 mM Mops (pH 7.4 with NaOH), 0.1 mM $CaCl_2$ and used for binding assay. Membrane proteins concentrations were determined according to the method of Lowry et al. [1951], using bovine serum albumin (BSA) as the standard.

Binding Studies

[^{3}H]Glibenclamide specific binding was determined by equilibrium binding assay. Radioligand binding assay was carried out by incubating [^{3}H]glibenclamide (0.4 nM) for 60 min at 25°C with human islet membranes (50–60 μg of proteins) in 1 ml of 50 mM Mops, pH 7.4 , 0.1 mM $CaCl_2$ buffer.

Incubation was stopped by addiction of ice-cold buffer and membrane-bound radioligand was collected onto Whatman GF/C glass fiber filters by vacuum filtration. The radioactivity trapped on the filters was counted using an 1600 TR scintillation counter. Nonspecific binding was determined in the presence of 1 μM glibenclamide.

Saturation analysis were carried out using increasing [^{3}H]glibenclamide concentrations (0.1–26 nM). In order to study the association kinetics, the binding reactions were started by the addition of [^{3}H]glibenclamide ligand (0.4 nM) and stopped after the indicated periods by rapid filtration.

Displacement studies were performed using the following oral hypoglycemic compounds: glimepiride, glibenclamide, tolbutamide, chlorpropamide (all of the sulfonylurea class) and metformin (a biguanide agent). Stock solution of unlabelled drugs were prepared in DMSO and diluted with assay buffer so that the final solvent concentration had no effects on the binding.

Insulin Secretion Studies

These studies were performed according to the procedures usually employed in our laboratory [Marchetti et al., 1994, 1995; Giusti et al., 1997]. Within 8–10 days from the isolation (during which the cells had been kept at 37°C in M199 culture medium containing 5.5 mM glucose), groups of approximately 10 islets of comparable size (100–150 μm in diameter) were preincubated at 37°C for 45 min, in Krebs–Ringer–bicarbonate solution (KRBS), supplemented with 3.3 mM glucose and 0.5% BSA. The islets were then washed and incubated at 37°C for 45 min in KRBS, containing 3.3 mM glucose plus 10 μM of either glibenclamide (a gift from Laboratori Guidotti, Pisa, Italy), glimepiride (a gift from Hoechst-Marion-Roussel), tolbutamide, chlorpropamide (both purchased from Sigma Chemical Co., Milan, Italy), or metformin (a gift from Laboratory Guidotti). Tolbutamide, chlorpropamide, and metformin were also tested at 200 μM concentration. Insulin levels at the end of the incubation periods were measured by a commercially available insulin IRMA kit (Medgenix, Brussels, Belgium).

Analysis of Results

Statistical analysis and curve-fitting were carried out on an IBM-compatible personal computer, using the nonlinear multipurpose curve-fitting program KINETICS, EBDA, and LIGAND [McPherson, 1985], from which the values of K_{ob}, the dissociation constant (K_d) and the maximum number of receptor sites (Bmax) were generated. Accordingly, a partial F test ($P < 0.01$) was used to determine whether the binding data were best fitted by a one or two-site model. IC_{50} values were derived by semilog plots of data from ligand displacement experiments. The Cheng–Prusoff equation was used to calculate K_i values [Cheng–Prusoff et al., 1973]. Values represent the means ±SEM derived from (n) experiments conducted in triplicate. Comparison of insulin secretion data between the varying groups was made by analysis of variance (ANOVA) and the Bonferroni test.

RESULTS

Figure 1 shows a typical appearance of isolated human islets, demonstrating the purity and morphological integrity of our islet preparations.

When membrane preparations from the purified islets were incubated in the presence of increasing concentrations of [^{3}H]glibenclamide, a saturable and high-affinity specific binding was detected that was protein, time, and temperature dependent.

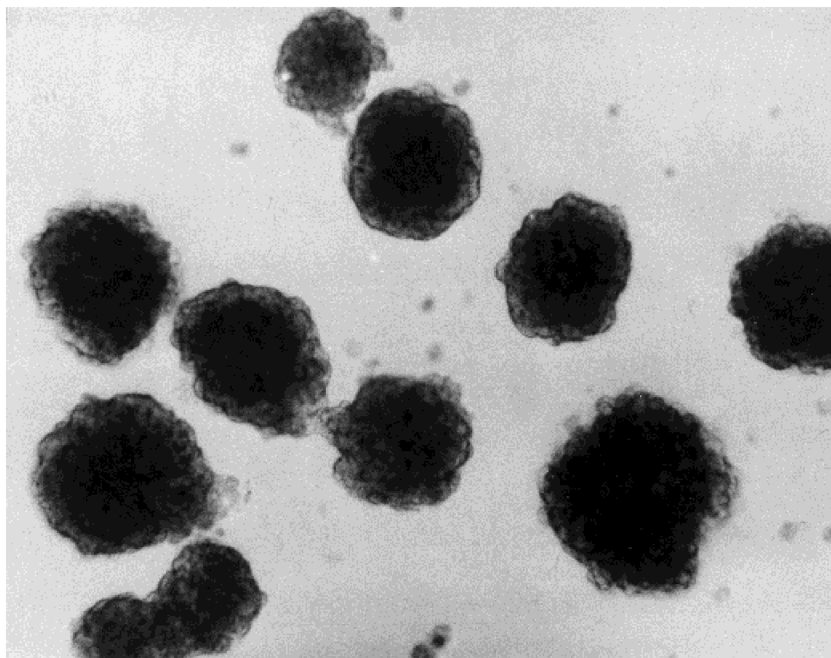

Fig. 1. Light photomicrograph of isolated human pancreatic islets.

Protein dependence was studied by incubating various amounts of crude human islet membranes with 0.4 nM [^{3}H]Glibenclamide for 60 min at 25°C. The extent of binding was proportional to the protein concentration of 15–250 µg/tube (Fig. 2). For all subsequent experiments with crude membrane preparations, a protein concentration in the linear range (50 µg/tube) was used.

The [^{3}H]glibenclamide binding was rapid, reached a maximum after 60 min of incubation at 25°C and remained stable for at least 120 min (Fig. 3). The exponential slopes of the pseudo-first-order kinetics, Kobs, calculated according to the McPherson program (1985) was 0.085 min^{-1}. A 60-min incubation time was selected for equilibrium studies of the interaction between the radioligand and the binding sites.

The study of temperature dependence of specific [^{3}H]glibenclamide binding demonstrated that the bound fraction decreased at 0°C or 37°C versus the values obtained at 25°C (Fig. 4).

Scatchard analysis of [^{3}H]Glibenclamide saturation curve showed the presence of a single population of high-affinity binding sites with a dissociation constant (K_d) of 1 ± 0.07 nM and a maximum number of binding sites (Bmax) of 657 ± 48 fmol/mg of proteins. Scatchard plots of the binding data were linear, consistent with the interpretation that an homogeneous population of binding sites was present in islet preparations ($P < 0.01$) (Fig. 5).

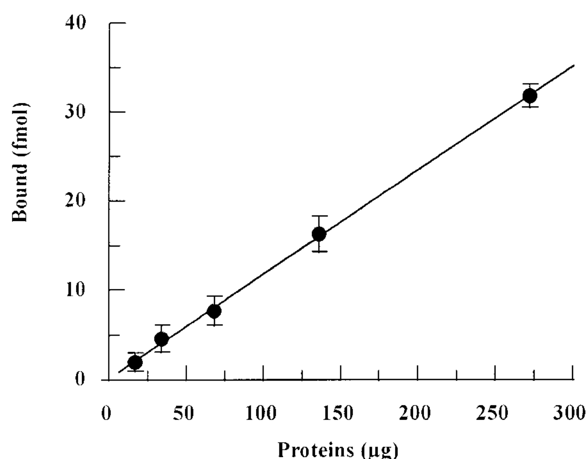

Fig. 2. Protein dependence of [^{3}H]glibenclamide binding to membranes. Various concentration of human islets membranes (15–250 µg/tube) were incubated with 0.3 nM [^{3}H]glibenclamide in the presence or absence of 1 µM unlabeled glibenclamide at 25°C for 60 min. Data points indicate mean ±SEM of results of three experiments performed in triplicate.

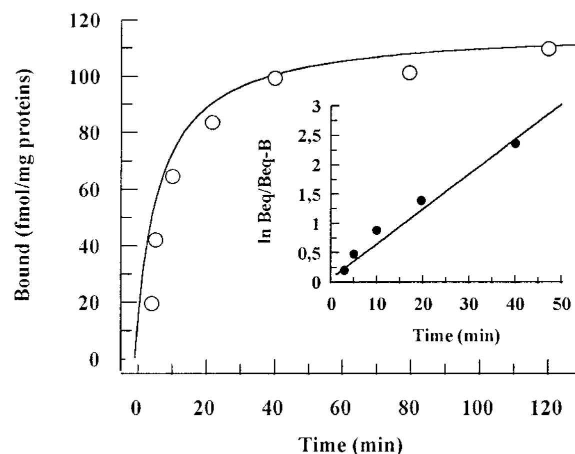

Fig. 3. Kinetics of [^{3}H]glibenclamide (0.4 nM) binding to human islets membranes (50 µg/tube) with association curve representative of a single experiment. Inset: First-order plots of [^{3}H]glibenclamide binding. Beq, amount of [^{3}H]glibenclamide bound to equilibrium; B, amount of [^{3}H]glibenclamide bound to each time. Computer analysis demonstrated that association data fit a one-component model significantly better than a two component model ($P > 0.05$). Association rate constant (K_{obs}) was 0.085 min^{-1}.

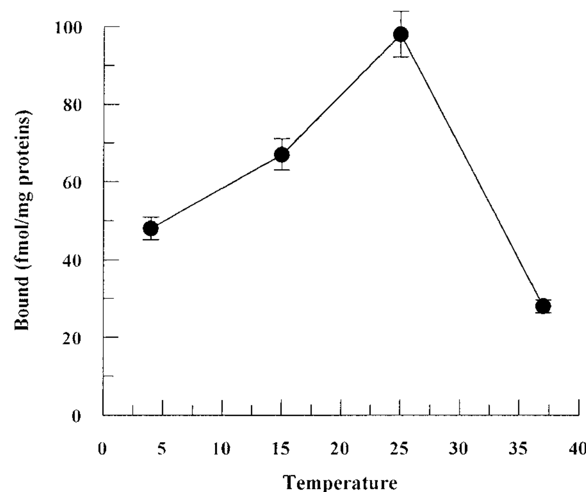

Fig. 4. Temperature dependence of [^{3}H]glibenclamide binding (0.4 nM) to membranes prepared from human islets. Assay was carrried out for 60 min at indicated temperature as described under Materials and Methods. Data points indicate mean ±SEM of results of three experiments performed in triplicate.

To test the structural requirements for [^{3}H]glibenclamide receptor binding, we examined the ability of several sulfonylurea compounds and of the biguanide derivative metformin, to inhibit the [^{3}H]glibenclamide binding to islet membranes.

Competition experiments results were showed in Table 1. The tritiated ligand was effectively displaced by low concentrations of both gliben-

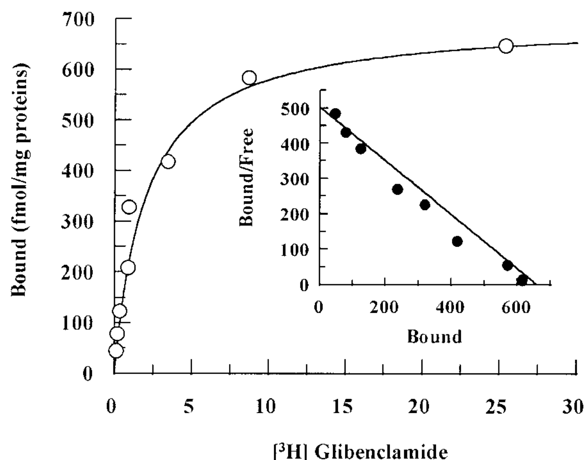

Fig. 5. Saturation curve of [^{3}H]glibenclamide to human islets membranes (○). Membranes were incubated for 60 min with eight concentrations of radioligand within a range of 0.1–26 nM. Details of binding procedure are described under Materials and Methods. Curve is representative of a single experiment. Inset: Scatchard plot of the saturation curve of [^{3}H]glibenclamide-specific binding to human membranes (●).

TABLE I. Specificity of [^{3}H]Glibenclamide Binding in Human Islet Membrane Preparations[a]

Compound	K_i (nM) ± SEM
Glibenclamide	1.20 ± 0.08
Glimepiride	1.60 ± 0.11
Tolbutamide	9,000 ± 620
Chlorpropamide	44,560 ± 617
Metformin	nd

[a]Five to eight concentration of displacers were examined. Each point is the mean ± SEM of four determinations. Average estimated K_i (inhibition constant) were calculated from IC_{50} (concentration inhibition 50%) values using the Cheng-Prusoff equation (1973).

clamide and glimepiride. Both ligands showed a similar rank order of potency with a K_i value of 1.0 ± 0.08 and 1.60 ± 0.12 nM, respectively. Tolbutamide and clorpropamide gave an affinity constant of 9 ± 0.6 μM and 44.56 ± 3.56 μM, respectively. [^{3}H]glibenclamide binding to islet membranes was not dispaced by 10 μM metformin.

Insulin release from the isolated islets at 3.3 mM glucose was significantly potentiated by the addition of 10 μM of either glibenclamide or glimepiride (Table 2). Tolbutamide and chlorpropamide had a potentiating effect when only used at 200 μM concentration. Metformin did not cause any significant change in insulin release at the glucose concentration used in this study.

TABLE II. Insulin Secretion from Human Islets at 3.3 mM Glucose (3.3 G) and Varying Antidiabetic Compounds

Secretagogue(s) 3.3 G	n	Insulin release (μU/ml)*
Alone	9	5.3 ± 0.6
+10 μM glibenclamide	12	13.5 ± 1.1**
+10 μM glimepiride	10	11.7 ± 0.8**
+10 μM tolbutamide	8	5.9 ± 0.5
+10 μM chlorpropamide	6	5.7 ± 0.4
+10 μM metformin	9	5.2 ± 0.4
+200 μM tolubtamide	9	12.1 ± 1.2**
+200 μM chlorpropamide	6	10.2 ± 0.9**
+200 μM metformin	10	5.8 ± 0.5

*$P < 0.01$ by ANOVA and **$P < 0.02$ vs. 3.3 G alone by the Bonferroni test.

DISCUSSION

The present report describes the characteristics of the sulfonylurea receptors from purified human pancreatic islet membranes for the first time. Sulfonylureas close an ATP-sensitive K^+ channel in the plasma membrane of the pancreatic β cells, thus allowing the occurrence of the chain of events leading to the insulin release [Yalow et al., 1960]. The initial event is the binding of the sulfonylureas to specific and saturable sites. These sites have been evidenced by autoradiography of tissue sections exposed to ^{3}H- or ^{125} I-labelled sulfonylureas [Aguilar-Bryan et al., 1990; Kramer et al., 1995, 1994] or by radioligand binding assays on laboratory animal derived preparations and cell lines [Aguilar-Bryan et al., 1990; Schmid-Antomarchi et al., 1987; Panten et al., 1989; Gaines et al., 1988].

[^{3}H]glibenclamide binding has been described also in cardiac [Miller et al., 1991; Gopalakrishan et al., 1991], smooth muscle cells [Zini et al., 1991; Kovacs et al., 1991] and in brain microsomes [Robertson et al., 1990]. A K_d value of 0.05–10 nM has been reported. Low-affinity [^{3}H]glibenclamide binding sites (K_d value of 100–400 nM) have also been described in β-cell tumor membranes and in other central and peripheral animal tissues, although the high-affinity binding site has been considered as the functional receptor [Martz et al., 1989; French et al., 1990].

We have now shown the presence of a single high-affinity [^{3}H]glibenclamide binding sites population in human islet membranes.

The binding was saturable, reversible, and protein concentration dependent. The affinity constant value that we found with human pancreatic islets (1 ± 0.07 nM) was comparable to that reported with rat islets in the pancreatic β-cell lines RIN 5 mF and HIT T15 (0.3–1.1 nM). We also observed that with human islets, the Bmax value (maximum density of [^{3}H]glibenclamide binding sites) was 657 ± 48 fmol/mg of proteins. Again, this value was within the range of those reported for β-cell tumor lines and rat islets membranes (546–1,000 fmol/mg of proteins).

Kinetics experiments showed that [^{3}H]glibenclamide binding to human islets was fast and reached equilibrium after 60 min. The monophasic nature of the time course of [^{3}H]glibenclamide binding pointed to the occurence of a bimolecular reaction involving only one type of binding centre. The exponential slope of the first-order kinetics, K_{obs}, were 0.085 min^{-1}. Conversely, with tumor β-cell lines a biphasic shape of the binding time course is consistently observed, suggesting the presence of either heterogeneous sulfonylurea binding sites or cooperativity between two (or more) similar binding sites in this particular experimental model.

We also assessed the specificity of the sulfonylurea receptors by comparing the ability of several unlabelled antidiabetic agents to displace [^{3}H]glibenclamide binding. There is general agreement that K_i values of sulfonylureas correlate well with their ability to close the β cell K-ATP channels and to stimulate insulin secretion [Boyd et al., 1988, 1991; Kaubisch et al., 1982; Geisen et al., 1985; Schmid-Antomarchi et al., 1987; Gaines et al., 1988; Panten et al., 1989]. This has been now demonstrated for the first time with human islets. We showed a rank order of binding potency for the tested compounds that was glibenclamide = glimepiride > tolbutamide > chlorpropamide ≫ metformin.

It is not surprising that these results were parallel with the insulinotropic potencies of the agents. Indeed, at 3.3 mM glucose, glibenclamide and glimepiride caused a potentiation of insulin release when used in the low micromolar range, whereas tolbutamide and chlorpropamide potentiated insulin secretion at higher concentration. On the other hand, metformin exerts its antidiabetic action by acting mainly at extrapancreatic sites.

REFERENCES

Aguilar-Bryan L, Nelson DA, Vu QA, Humphrey MB, Boyd III AE (1990): Photoaffinity labeling and partial purification of the β-cell sulfonylurea receptor using a novel biologically active gliburide analog. J Biol Chem 265: 8218–8224.

Ashcroft FM, Harrison DE, Ashcroft SJH (1984): Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 312:446–448.

Boyd AE III(1988): Sulfonylurea receptors, ion channels and fruit flies. Diabetes 37:847–850.

Boyd AE III, Aguilar-Bryan L, Bryan J, Kunze DL, Moss L, Nelson DA, Rajan AS, Raef H, Xiang H, Yaneyn GC (1991): Sulfonylurea signal trasduction. Recent Prog Horm Res 47:299–317.

Cheng Y, Prusoff WH (1973): Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108.

Duckworth WC, Solomon SS, Kitabchi AE. (1972): Effect of chronic sulfonylurea therapy on plasma insulin and proinsulin levels. J Clin Endocrinol. Metab 35:585–591.

French JF, Riera LC, Mullins UL, Sarmiento JC (1991): Modulation of [^{3}H]glibenclamide binding to cardiac and insulinoma membranes. Eur J Pharmacol 207:23–28.

Gaines KL, Hamilton S, Boyd AE III (1988): Characterization of the sulfonylurea receptor on beta-cell membranes. J Biol Chem 263:2589–2592.

Geisen K, Hitzel V, Okomonopoulos R, Punter J, Weyer R, Summ HD (1985): Inhibition of [^{3}H]glibenclamide binding to sulfonylurea receptors by oral antidiabetics. Arzneim Forsch 35:707–712.

Giusti L, Marchetti P, Trincavelli L, Lupi R, Martini C, Lucacchini A, Del Guerra S, Tellini C, Carmellini M, Navalesi R (1997): Peripheral benzodiazepine receptors in isolated human pancreatic islets. J Cell Biochem 64: 273–277.

Gopalakrishnan M, Johnson DE, Janis RA, Triggle DJ (1991): Characterization of binding of the ATP-sensitive potassium channel ligand, [^{3}H]glibenclamide, to neuronal and muscle preparation. J Pharmacol Exp Ther 257: 1162–1171.

Grodsky GM, Epstein GH, Franska R (1972): Pancreatic action of the sulfonylureas. Fed Proc 31:2714–2719.

Gylfe E, Hellman B, Sehlin J, Taljedal LB (1984): Interaction of sulfonylureas with the pancreatic β-cell. Experientia 40:1126–1134.

Kaubisch N, Hammer R, Wolheim C, Renold AE, Offord RE (1982): Specific receptors for sulfonylureas in brain and in a β-cell tumor of rat. Biochem Pharmacol 31:1171–1174

Kovacs RJ, Nelson MT (1991): ATP-sensitive K^+ channels from aortic smooth muscle incorporated into planar lipid bilayers. Am J Physiol 261:H604–H609.

Kramer H, Muller G, Girbig F, Gutjar U, Kowaleski S, Hartz D, Summ HD (1994): Differential interaction of glimepiride and glibenclamide with the β-cell sulfonylurea receptor. II. Photoaffinity labeling of a 65 KDa

protein by [^{3}H]Glimepiride. Biochim Biophys Acta 1191: 267–277.

Kramer W, Muller G, Girbig F, Gutjahr U, Kowalewski S, Hartz D, Summ HD (1995): The molecular interaction of sulfonylurea with β-cell ATP sensitive K^+-channels. Diabetes Res Clin Pract 28(suppl):S67–S80.

Lebovitz HE, Feinglos MN, Bocholtz HK (1977): Potentiation of insulin action: a probable mechanism for the anti-diabetic action of sulfonylurea drugs. J Clin Endocrinol Metab 45:601–604.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the Folin phenol reagent. J Biol Chem 263:289–2592.

Marchetti P., Giannarelli, Velani G, Andreazzi M, Cruschelli L, Cosimi S, Viacava P, diCarlo A, Bevilacqua G, Navalesi R. (1994): Collagenase distension, to-step sequenzial filtration, and Histopaque gradient purification for consistent isolation of pure pancreatic islets from the market-age (6-month-old) pig. Transplantation 57:1532–1535.

Marchetti P, Giannarelli R, Cosimi S, Masiello P, Coppelli A, Viacava P, Navalesi R. (1995): Massive isolation, morphological and functional characterization, and xenotransplantation of bovine pancreatic islets. Diabetes 44: 375–381.

Martz A, Jo I, Jung CY (1989): Sulfonylurea binding to adipocyte membranes and potentiation of insulin-stimulated hexose transport. J Biol Chem 264:3672–13678.

McPherson GA (1985): A collection of radioligand binding analysis programs. J Pharmacol Methods 14:213–228.

Miller JA, Velajo NL, Dage RC, Rampe D (1991): High-affinity [^{3}H]Glibenclamide binding sites in rat neuronal and cardiac tissue: Localization and developmental characteristics. J Pharmacol Exp Ther 256:358–364.

Niki I, Ashcroft FM, Ashcroft SJH (1989): The dependence of intracellular ATP concentration of ATP-sensitive K-channels and of Na,K-ATPase in intact HIT-T15 beta-cells. FEBS Lett 257:361–364.

Niki I, Kelly RP, Ashcroft SJH, and Ashcroft FM (1989): ATP-sensitive K-channels in HIT T15 β cells studied by patch-clamp methods, ^{86}Rb efflux and glibenclamide binding. Pflugers Arc. 415:47–55.

Niki I, Nicks J L, Ashcroft SJH (1990): The beta-cell glibenclamide receptor is an ADP-binding protein. Biochem J 268:713–718.

Ozanne SE, Guest PC, Hutton JC, Hales CN (1995): Intracellular localization and molecular heterogeneity of the sulfonylurea receptor in insulin-secreting cells. Diabetologia 38:277–282.

Panten U, Burgfeld J, Goerke F, Rennicke M, Schwanstecher M, Wallasch A, Zunkler BJ, Lenzen S (1989): Control of insulin secretion by sulfonylureas, meglitimide and diazoxide in relation to their binding to the sulfonylurea receptor in pancreatic islets. Biochem Pharmacol 38:1217–1229.

Robertson DW, Schober DA, Krushinski JH, Mais DE, Thompson DC, Gehlert DR (1990): Expedient synthesis and biochemical properties of an [^{125}I]-labelled analogue of gliburide, a radioligand for ATP-inhibited potassium channels. J Med Chem 33:3124–3126.

Schmid-Antomarchi H, De Weille J, Fosset M, Lazdunski M (1987): The antidiabetic sulfonylurea glibenclamide is a potent blocker of the ATP-modulated K^+ channel in insulin secreting cells. Biochem Biophys Res Commun 146:21–25.

Schmid-Antomarchi H, De Weille J, Fosset M, Lazdunski M (1987): The receptor for the antidiabetic sulfonylureas controls the activity of the ATP-modulated K^+-channel. J Biol Chem 262:14840–15844.

Sturgess NC, Ashcroft MIJ, Cook DI, Hales CN (1985): The sulfonylurea receptor may be an ATP-sensitive potassium channel. Lancet 2:474–475.

Yalow RS, Black H, Villazon M, Berson S (1960): Comparison of the plasma insulin levels following administration of tolbutamide and glucose. Diabetes 9:356–362.

Zini S, Yehezkel BA, Ashford MJL (1991): Characterization of sulfonylurea receptors and the action of potassium channel openers on cholinergic neurotransmission in guinea pig isolated small intestine. J Pharmacol Exp Ther 259:566–573.